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Bacterial Component Induced Inflammatory Response in Roosters from Diverse Genetic Lines

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Summary and Implications

Lipopolysaccharide (**LPS**) is a component of gram-negative bacterial cell walls that can stimulate a cellular inflammatory reaction. The objective of this study was to determine the effect of genetic line and time on LPS-induced pro-inflammatory response. Thirty roosters were divided into two groups of challenge and non-challenge for this experiment. The challenged birds were intravenously injected with LPS, to characterize the genetic response to inflammatory stimuli. At hours one and three post-injection, blood was collected, white blood cells (**WBC**) were isolated, and RNA isolated from the WBC. Real-time quantitative polymerase chain reaction (RT-**qPCR**) was conducted to estimate gene expression levels from mRNA. In summary, all three genetic lines responded to LPS with enhanced gene expression, and the three unique genetic lines differed both in baseline levels and in LPS response in mRNA expression of IL-6 and IFN-γ cytokines. The results indicate that there is a genetic component associated with inflammation response.

Introduction

Lipopolysaccharide can induce an inflammatory reaction, though it does not have the added complications such as inducing disease and bio-security precautions that comes with live bacteria. Lipopolysaccharide from *Salmonella typhimurium* was used in this study to induce an inflammatory reaction. Inflammation is an immunological pathway that takes place starting at the cellular level while simultaneously causing visual effects such as redness, pain, heat, and swelling. The pro-inflammatory stage takes place in the earlier phases of inflammation. Pro-inflammatory genes were the focus to look at early and later stages of inflammation, interleukin-6 (**IL-6**) and interferon-γ (**IFN-γ**), respectively. The main function of IL-6 is to aid in T- and B- cell maturation and differentiation and induces a fever; interferon-γ increases expression of the major histocompatibility complex and activates macrophages. To better understand gene expression levels across breed, three different lines, broiler, Leghorn, and Fayoumi, were studied.

Materials and Methods

Animals

A total of 30 adult male breeder birds were used, with three different lines (10 birds per line) tested, broiler (br), Fayoumi (M), and Leghorn (GHs). Each group of 10 birds were split into two different groups, one group (n=6) with LPS injection, challenged, and the other group (n=4) with saline (**PBS**) injection, non-challenged. In order to facilitate data collection, injections were replicated over the course of three days, 10 birds per day, one injection per bird. The amount of LPS given to each bird was at a dose of 0.1mg of LPS/kg of body weight. At 1h and 3h post-injection, approximately 5ml of blood was collected from each bird for RNA isolation. At post-injection hours 0, 1, 2, 3, 5, and 7 body temperatures were measured cloacally from each of the birds to examine whether the birds expressed a fever with being challenged compared to those non-challenged.

White blood cell isolation and RNA isolation

White blood cells were isolated from the blood using a discontinuous gradient. The white blood cells were collected and stored in RNAlater overnight to stabilize and protect the RNA. The RNA isolations were completed using RNAqueous Kit from Ambion.

Quantitative PCR

Quantitative polymerase chain reaction (**qPCR**) was utilized to measure the expression levels of the aforementioned genes of interest, IL-6 and IFN-γ.

Statistical Analysis

The software package, JMP Pro 10 was used to analyze the data.

Results and Discussion

Genetic line had a significant effect on IL-6 (P= 0.014) and IFN-γ (P=0.029) expression levels. The RNA expression levels for IL-6 were significantly higher in broilers when compared to Leghorns (Figure 1). The RNA expression levels for IFN-γ were significantly higher in Leghorns when compared to Fayoumis (Figure 2), with the broilers being numerically closer to the Leghorns. These differences among lines in RNA expression levels for IFN-γ and IL-6 indicate a genetic component is involved in immune response.

Furthermore, LPS versus PBS was statistically different for IL-6 (P= 0.004) and IFN-γ (P=0.004) with LPS having higher levels of RNA expression levels than PBS. This study shows that LPS induced a pro-inflammatory inflammation response; by activation of IL-6 and IFN-γ (Figure 3; Figure 4).

Body temperature was significant at time PI 1 for LPS (P=0.001), time 2 for line (P=0.001), and time 3 and 7 for line*LPS (P= 0.009 and P= 0.027, respectively). In both challenged and non-challenged birds, broilers and Fayoumis showed the most similarities between body temperatures when comparing the three lines (Figure 5). Broilers and Fayoumis rose and dropped in body temperature at the same time points while the Leghorns declined in body temperature at an earlier time point. This suggests that broilers and Fayoumis demonstrate similar immune responses when converting their energy from normal metabolism to responding to an immune response.

In conclusion, this study shows that *Salmonella typhimurium* LPS induced an inflammatory response and distinct genetic lines respond differently to immune challenges.

Figure 1. Effect of Line on RNA Expression Levels for IL-6. Where Br=Broiler, GHs=Leghorn, M=Fayoumi.

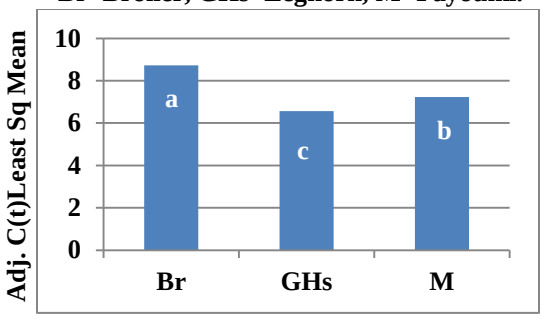

Figure 2. Effect of Line on RNA Expression Levels for IFN-γ. Where Br=Broiler, GHs=Leghorn, M=Fayoumi.

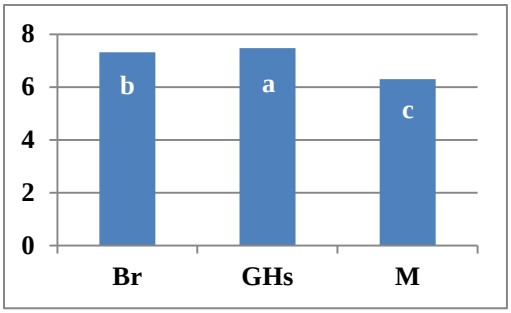

Figure 3. Effect of LPS on RNA Expression Levels for IL-6.

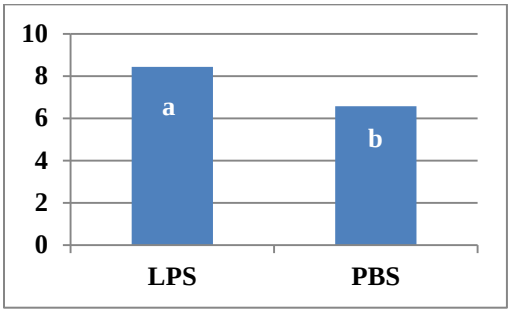

Figure 4. Effect of LPS on RNA Expression Levels for IFN-γ.

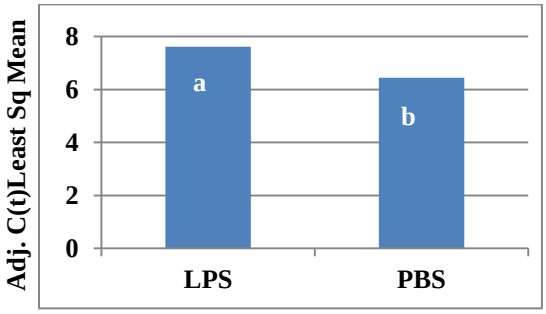

Figure 5. Body temperatures for Broilers, Leghorns, and Fayoumis challenged and non-challenged at hours 0, 1, 3, and 7 post injection for Line*LPS. Where Br=Broiler, GHs=Leghorn, M=Fayoumi.